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Choroideremia and Deafness with Stapes Fixation: A Contiguous Gene Deletion Syndrome in Xq21

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Summary

The study of contiguous gene deletion syndromes by using reverse genetic techniques provides a powerful tool for precisely defining the map location of the genes involved. We have made use of individuals with overlapping deletions producing choroideremia as part of a complex phenotype, to define the boundaries on the X chromosome for this gene, as well as for X-linked mixed deafness with perilymphatic gusher (DFN3). Two patients with deletions and choroideremia are affected by an X-linked mixed conductive/sensorineural deafness; one patient, XL-62, was confirmed at surgery to have DFN3, while the other patient, XL-45, is suspected clinically to have the same disorder. A third choroideremia deletion patient, MBU, has normal hearing. Patient XL-62 has a cytogenetically detectable deletion that was measured to be 7.7% of the X chromosome by dual laser flow cytometry; the other patient, XL-45, has a cytogenetically undetectable deletion that measures only 3.3% of the X chromosome. We have produced a physical map of the X-chromosome region containing choroideremia and DFN3 by using routine Southern blotting, chromosome walking and jumping techniques, and long-range restriction mapping to generate and link anonymous DNA sequences in this region. DXS232 and DXS233 are located within 450 kb of each other on the same *Sfi*I and *Mlu*I fragments and share partial *Sal*I fragments of 750 and >1,000 kb but are separated by at least one *Sal*I site. In addition, DXS232, which lies outside the MBU deletion, detects the proximal breakpoint of this deletion. We have isolated two new anonymous DNA sequences by chromosome jumping from DXS233; one of these detects a new *Sfi*I fragment distal to DXS233 in the direction of the choroideremia gene, while the other jump clone is proximal to DXS233 and detects a new polymorphism. These data refine the map around the loci for choroideremia and for mixed deafness with stapes fixation and will provide points from which to isolate candidate gene sequences for these disorders.

Introduction

Choroideremia (MIM 30310) is an X-linked retinal dystrophy of unknown pathogenesis (McCulloch and McCulloch 1948; Sorsby et al. 1952). Affected males have peripheral retinal disease with progressive night blindness and visual field constriction in the first 2 decades of life and severe visual impairment by the fourth to fifth decade. Ophthalmoscopic examination in affected males shows a progressive atrophy of the retinal pigment epithelium and choriocapillaris. Heterozygous females are generally asymptomatic but do have characteristic patchy retinal pigment epithelial mosaicism, reflecting random X inactivation.

The locus for choroideremia has been mapped to the Xq13-q21 region by tight linkage to RFLP markers DXYS1, DXYS12, and DXS72 (Nussbaum et al. 1985; Gal et al. 1986; Schwartz et al. 1986; Lesko et al. 1987;

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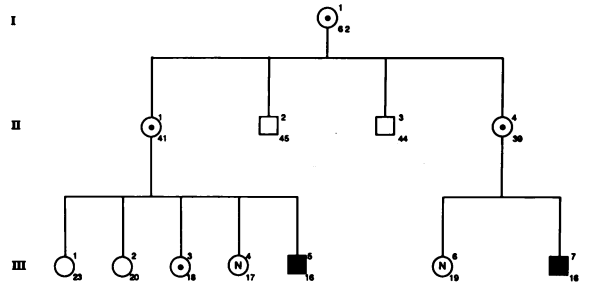


Figure 1 Pedigree of kindred XL-62. Numbers at the lower right of each figure indicate the present age of the individual. Ⓝ = normal female by exam; ⊙ = carrier of choroideremia by exam; ■ = male with choroideremia and mixed deafness with perilymphatic gusher; □ = normal male by history.

MacDonald et al. 1987; Sankila et al. 1987). This map position has been supported by the identification of individuals with deletions and translocations in Xq21 who also have choroideremia (Rosenberg et al. 1986; Schwartz et al. 1986; Hodgson et al. 1987; Nussbaum et al. 1987; Cremers et al. 1988, 1989; Schwartz et al. 1988). In these individuals, Xq21 deletions and rearrangements are associated with various complex syndromes that include mental retardation, deafness, seizures, and multiple congenital anomalies. In addition, a few patients with choroideremia alone have apparently normal karyotypes but are deleted for an anonymous DNA locus, DXS165, which maps to the region Xq21.1-Xq21.2 (Cremers et al. 1987). One female with the disease was recently found to have an X;13 translocation with the breakpoint on the X chromosome in Xq21 (Siu et al. 1988).

Patients with choroideremia and chromosomal abnormalities involving Xq21 are useful not only for more precise definition of the map position of this disease but also for the identification and localization of other disease genes located within these contiguous gene deletions. Patients with large deletions or duplications that include Xq21 have been reported with choroideremia, severe psychomotor retardation, and multiple congenital malformations including cleft lip and palate (Tabor et al. 1983; Rosenberg et al. 1986). With smaller deletions, a more limited clinical phenotype is seen. In one kindred (Hodgson et al. 1987), the proband had an Xq21 interstitial deletion and moderate mental retardation, choroideremia, and an unusual EEG but no hearing impairment. In another kindred, reported by Ayazi (1981), a submicroscopic interstitial deletion of Xq21 was documented (Nussbaum et al. 1987) in probands with choroideremia, moderate mental retardation, and congenital mixed (sensorineural and conductive) deafness. Thus, males with these interstitial deletions have complex phenotypes, which may include choroideremia, congenital mixed deafness, and cleft lip and palate, all disorders known to be inherited as single gene X-linked traits in at least some families.

We describe our further studies localizing the genes for choroideremia and a form of X-linked deafness with respect to physical landmarks such as cloned DNA sequences, deletion breakpoints, and rare-cutting enzyme sites by using a combination of deletion mapping, flow karyotyping, chromosome jumping, and long-range restriction mapping.

Material and Methods

Kindreds

Kindred XL-62.—The pedigree of this previously unreported kindred is shown in figure 1. The proband, individual III-7, is a 16-year-old male who first presented in infancy with preverbal deafness and delayed development. Childhood was complicated by chronic and recurrent otitis media. There was no history of seizures.

When first evaluated at age 13 years by one of us (M.L.), he was found to be a nondysmorphic, retarded male with weight 27 kg (below the fifth percentile), height 132 cm (below the fifth percentile), and head circumference 55 cm (75th percentile). Neurological examination was normal, and EEG showed nonspecific slowing without epileptiform discharges. Developmental assessment using testing appropriate for deaf children revealed that he was functioning at a 3-year-old level. Ophthalmological examination showed characteristic findings of choroideremia. A chromosomal analysis showing 46, del(X)(q21)Y has been published previously (Nussbaum et al. 1987). Audiometric evaluation, at a time when tympanograms and otolaryngological examinations were normal and no middle ear effusion was present, revealed a moderately severe mixed hearing loss bilaterally.

At age 13 years, he underwent left-middle-ear exploration and stapedectomy, during which he was found to have stapes fixated to the foot plate with a patent cochlear aqueduct; on stapedectomy, he had marked perilymphatic gusher.

The proband's cousin, individual III-5 (fig. 1), is currently 16 years old. He was unresponsive to sound and developmentally delayed in the first year of life. Childhood was complicated by breathing difficulty and stridor due to tracheal malacia, but there was no history of chronic otitis or seizures. Developmental assessment

at age 10 years was interpreted as being at the 7 3/12-year level in comparison with deaf peers.

When evaluated at age 12 2/12 years by one of us (M.L.), he was a mildly but nonspecifically dysmorphic retarded male with growth failure: his height was 130 cm (below the fifth percentile), his weight was 26 kg (below the fifth percentile), and head circumference was 55 cm (75th percentile). General physical and neurological examinations were normal. Head CT scan was normal; EEG showed diffuse slowing without epileptiform activity. Repeated developmental assessments were scattered in the 4 1/2–8-year-old level in comparison with deaf peers. Ophthalmological examination was characteristic of choroideremia. Chromosomal analysis was 46, del(X)(q21)Y, identical to that of his cousin. Otolaryngological and audiological assessment showed severe-to-profound mixed hearing impairment in both ears, despite normal tympanic membrane compliances and middle-ear pressures and the absence of middle-ear effusion.

Family evaluation, including ophthalmological examinations and karyotype analyses, identified both mothers and the maternal grandmother (individuals II-1, II-4, and I-1) of the two affected males as carriers of choroideremia and carriers of the Xq21 deletion, as was the sister (individual III-3) of one of the affected boys. In addition, II-1 showed mild high-frequency sensorineural hearing loss bilaterally.

Kindred XL-45.—This kindred was first reported in 1981 by Ayazi. The proband (case 1 in that report), currently 39 years old, has had deafness since infancy and night blindness that developed during childhood. A diagnosis of choroideremia was first made at age 30 years. Audiological evaluations revealed severe-to-profound sensorineural hearing loss, although he derived some benefit from a hearing aid. When examined by two of us (R.L.N. and R.A.L.) at age 36 years, he was an obese man of normal stature with normal physical and neurological examination except for his impairments of vision and hearing and his moderate mental retardation. Ophthalmoscopic examination was typical of choroideremia. His brother (case 2 in the 1981 report by Ayazi) is currently age 30 years. When examined by us (R.L.N. and R.A.L.), he had a similar general physical and neurological examination; ophthalmological examination showed choroideremia. Neither patient had a history of seizures. Their mother (case 4 in the report of Ayazi) showed the classical carrier retinal state for choroideremia.

Karyotype analysis of the probands in XL-45 appeared to be normal 46,XY although in some cells there was a suggestion of decreased size of the Xq21 band (Nussbaum et al. 1987).

Kindred of Hodgson et al.—This kindred was reported by Hodgson et al. in 1987. The proband was a 30-year-old man with mental retardation (IQ 67 at age 8 years), choroideremia, and an interstitial deletion of Xq21. Of note, however, is that he had no hearing impairment. In addition, he had an EEG described as "moderate diffuse abnormality of an unusual kind, with rhythmic components." A history of seizures was also obtained in all four female relatives of the proband who carried the Xq21 deletion. A lymphoblastoid cell line (MBU) was established from the proband in this kindred.

A total of 42 unrelated probands with isolated choroideremia have been identified and examined by one of us (R.A.L.).

Lymphocytes from heparinized blood samples from family members in kindreds XL-62 and XL-45 and from the probands with isolated choroideremia were transformed with Epstein-Barr virus in the presence of 10 μg/ml cyclosporine A. The cell line MBU is a transformed lymphoblastoid cell line from the proband in the kindred reported by Hodgson et al. GM1416, a lymphoblastoid cell line with 48,XXXX karyotype, was obtained from the Mutant Cell Repository, Institute for Medical Research.

DNA probes used in this study are as follows: pXPGK-RI0.9 (PGK), pX65H7 (DXS72), pJL77 (DXS276), pJL68 (DXS232), pJL8 (DXS233), pXG7c (DXS95), pDP34 (DXYS1), St25 (DXYS12), p19–2 (DXS3), and S21 (DXS17). Probe details have been recorded by Davies et al. (1987) and Mandel et al. (1988).

Southern Blot Analysis

High-molecular-weight DNA was isolated according to the method of Aldridge et al. (1984). DNA fragments were separated by electrophoresis in 0.8% agarose gels and transferred to nylon filters (Zetabind®) with 10 × SSC. Prehybridization and hybridization conditions have been described elsewhere (Boggs and Nussbaum 1984). DNA probes were uniformly labeled to a specific activity of 1×10^9 cpm/μg by the random priming method (Feinberg and Vogelstein 1983).

Phage Library Screening

Chromosome jumping was accomplished using a 100-kb jumping library (Collins et al. 1987) constructed in a modified Charon 3A phage vector. Approximately 3×10^6 clones were screened with each probe. In addition, a genomic library from the lymphoblastoid cell

line GM1416, constructed in the phage vector EMBL3, was used for chromosome walking and to isolate additional DNA around probes that did not initially identify clones in the jumping library. The construction of this library has been described elsewhere (Schnur et al. 1989). Approximately 1 × 10^6 clones from this library were screened. Phage were plated on the bacterial host strains MC1061 (jumping library) or NM539 (GM1416 genomic library). Phage clones were identified and plaque purified by the method of Benton and Davis (1977). Prehybridizations and hybridizations were carried out in 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 1% SDS, and 100 mg/ml herring sperm DNA at 42°C. Probes were prepared as radioactively labeled DNA made by the random priming technique (Feinberg and Vogelstein 1983) or as radioactively labeled RNA prepared from inserts subcloned into pGEM3-blue and transcribed with SP6 or T7 RNA polymerase (Promega Biotec). Hybridization was carried out for 12–24 h at 42°C. Filters were washed in 2 × SSC, 0.1% SDS at room temperature for 15 min, then at 65°C for 40 min. Miniprep phage DNA was prepared according to standard protocols (Maniatis et al. 1982). Phage clone inserts were subcloned into pGEM3-blue and used to transform the bacterial strain DH5α.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis was carried out using a contour-clamped homogeneous electric field (CHEF) apparatus (Chu et al. 1986). Lymphoblastoid cells from GM1416 and normal male cell lines were embedded in low-melt agarose (SeaPlaque®; FMC Corp.) at a final concentration of 2.5 × 10^7 cells/ml. Agarose plugs were made in tygon tubing (3/32-inch inner diameter). Plugs were treated with ESP (0.1 M EDTA, 1 mg/ml proteinase K, 1% N-lauroylsarcosine) for 48 h at 50°C, then were dialyzed extensively in large volumes of TE (10 mM Tris pH8, 1 mM EDTA). Restriction digests (of 1 cm plugs) were carried out for 16 h in a volume of 100 μl, using 40 units of enzyme. Plugs were then loaded directly into a 1% agarose gel and electrophoresed in 0.4 × TBE. Standard run conditions consisted of 13 V/cm for 40 h with an 80-s switching interval. Temperatures were maintained at 10°C. *Saccharomyces cerevisiae* chromosomes from strains YPH49 or YPH149 were used as molecular-weight markers. These were prepared according to the method of Schwartz and Cantor (1984).

Southern transfer of DNA to Zetabind was carried out in 10 × SSC following 15 min in 0.25 N HCl, 2 × 30 min in 0.5 M NaOH, 1.5 M NaCl, and 2 × 30 min in 3 M NaCl, 0.5 M Tris pH7.5. Transfer was allowed to take place for 20–24 h. Prehybridizations and hybridizations were carried out at 42°C in 50% formamide, 3 × SSPE, 1% SDS, 5 × Denhardt's solution, and 100 μg/ml herring sperm DNA. Hybridizations included 8% dextran sulfate and were carried out for 36–40 h. Filters were washed in 2 × SSC, 0.1% SDS at room temperature for 15 min, then at 65°C for 40 min. Autoradiographic exposures lasted 1–3 d.

Flow Karyotype Analysis

Metaphase chromosomes were isolated from lymphoblastoid cell lines, stained with Hoechst 33258 and chromomycin A3, and analyzed on a dual beam flow cytometer (van den Engh et al. 1985, 1988). The Hoechst and chromomycin fluorescence intensities of approximately 30,000 chromosomes were quantified. The peaks in the bivariate distribution—or flow karyotype—reflect both the relative DNA content and base composition of the chromosomes. An iterative bivariate Gauss distribution fitting procedure was used to objectively assign peak positions in flow karyotypes (G. van den Engh, D. Hanson, and B. Trask, unpublished data). To estimate deletion size, the peak position of the derivate X chromosome in XL-45 was compared with that of the normal X chromosome in the same karyotype. The flow karyotype of XL-62 was normalized (using the average Hoechst and chromomycin intensity of all autosomes except 9–12) to a set of karyotypes of unrelated individuals to compare the position of the deleted X chromosome in this male with the mean position of 37 normal X chromosomes (B. Trask, G. van den Engh, R. Nussbaum, C. Schwartz, and J. Gray, unpublished data).

Results

Southern Blot Analysis

The results of Southern blot analyses of DNA from XL-45, XL-62, and MBU, with a number of probes from the Xq13-q21 region, are summarized in table 1. The relative order of these markers is based on three sources. First, data previously published by us (Lesko et al. 1987) or by others (Cremers et al. 1988, 1989) have been used. Second, under the assumption that the deletions in these three patients are uncomplicated, contiguous deletions, some probes can be ordered because they are separated by breakpoints in the deletions. Finally, long-range restriction mapping has linked some markers on common restriction fragments, thereby establishing their physical proximity (*vide infra*). Note

Table 1

Proband/Phenotype	Loci									
	PGK	*DXS72*	*DXS276*	*DXS232*	*DXS233*	*DXS95*	*DXYS1*	*DXYS12*	*DXS3*	*DXS17*
XL-62/choroideremia, deafness	+	−	−	−	−	−	−	+	+	+
XL-45/choroideremia, deafness	+	+	+	−	−	−	+	+	+	+
MBU/choroideremia, no deafness	+	+	+	+	−	−	−	−	−	+

from table 1 that DXS233 and DXS95 are deleted in XL-45, XL-62 and MBU.

Flow Cytometry

To determine the absolute size of the deletions in the XL-62 and XL-45 probands, the DNA contents of derivative and normal X chromosomes were quantified by flow cytometry (B. Trask, G. van den Engh, R. Nussbaum, C. Schwartz, and J. Gray, unpublished data). In XL-62, ~7.8% of the X chromosome was found to be deleted relative to the mean DNA content of 37 normal X chromosomes in a set of unrelated individuals, giving an estimated deletion of ~12,000 kb on the basis of an assumption that the X chromosome contains 158,000 kb DNA. For XL-45, flow estimates of deletion size were made using a carrier female's cell line, comparing one X chromosome to the other. An estimated 3.5% of the X chromosome—~5,300 kb—was found to be deleted.

Long-Range Restriction Mapping

Given the relevance of DXS233 and DXS95 as markers that lie within the overlap of the XL-45 and MBU deletions (table 1), the region that contains the choroideremia locus, we have begun to generate a long-range restriction map around these two markers.

Figure 2*A* details the long-range restriction map constructed for the genomic region containing DXS232 and DXS233. Data obtained by sequentially hybridizing the same filters with pJL68 (DXS232) and pJL8 (DXS233) and with pJL8 and pCH2 (DXS368) (see chromosome jumping results) are shown in figure 3. pJL8 and pJL68 identify the same *Sfi*I fragments while detecting different *Sal*I fragments (fig. 3*A*). The same partial *Sal*I fragments of ~750 kb and >1,000 kb are, however, detected by both probes. The partial *Sal*I fragment of ~800 kb that is detected by pJL8 and not by pJL68 extends in the distal direction from pJL8. Minor differences in estimated fragment sizes between these results and those of Cremers et al. (1989) probably result from the use of different pulsed-field gel electrophoresis systems. Both probes also detect the same 850-kb *Mlu*I fragment (data not shown). Double digests of *Mlu*I/*Sfi*I and of *Sal*I/*Sfi*I allowed the placement of the distal *Mlu*I site and confirmation of the distal *Sal*I and *Sfi*I sites. The presence of DXS232 and the deletion of DXS233 in MBU (table 1) allows the centromere-telomere orientation of the map.

A smaller restriction map can be constructed around the locus DXS95 by using probe pXG7c (fig. 2*B*). The relative orientation of the *Sal*I and *Mlu*I fragments to the larger *Sfi*I fragment is not known. Also, the lack of additional nearby probes prevents the orientation of this map relative to the centromere. Filters hybridized with pXG7c were stripped and hybridized with pCH2 (see results below on chromosome jumping). No fragments could be shown to hybridize to both probes, preventing the linking of these two maps.

Detection of MBU Breakpoint

CHEF filters containing normal male, GM1416, and MBU DNAs were hybridized with pJL68 (DXS232) (fig. 4). While the 265-kb *Sal*I fragment detected in normal DNAs by pJL68 is unaltered in MBU, the 750-kb partial *Sal*I fragment that is detected by both pJL68 and pJL8 (DXS233) (see fig. 3) is missing in MBU DNA. The variation in migration of the 265-kb *Sal*I fragment seen in the MBU and XY lanes versus the 4X lane can be attributed to the overloading of the MBU and XY lanes relative to the 4X lane; this can be seen by ethidium bromide staining (data not shown). Two new partial fragments (350–500 kb) are detected in MBU DNA by pJL68. This result places the proximal MBU breakpoint at or distal to the *Sal*I site that is located between pJL8 and pJL68 (since the 265-kb *Sal*I fragment is unaltered) and pJL8. However, there remains a formal possibility that the breakpoint lies proximal to the *Sal*I site and that a 265-kb *Sal*I fragment is created coincidentally by the interstitial deletion. Either placement predicts that the 450-kb and 780-kb *Sfi*I fragments de-

tected by pJL68 should also be altered in MBU. As shown in figure 4, the 780-kb *Sfi*I fragment is absent from MBU DNA, while coincidentally, the new *Sfi*I fragment spanning the deletion breakpoints is close in size to the original 450-kb fragment.

CHEF filters containing normal male, GM1416, and XL-45 DNAs were hybridized with pDP34 (DXYS1). No alteration in the 450-kb *Sfi*I fragment detected by this probe was seen in XL-45 DNA (data not shown).

Chromosome Jumping from DXS233

To further characterize the region in the XL-45 deletion containing the choroideremia locus, we have begun chromosome walking and jumping experiments from DXS233.

An initial screen of the jumping library with probe pJL8 provided no positive clones. Consequently, the GM1416 genomic library was screened with pJL8 to isolate more unique-sequence DNA surrounding this probe. One million recombinant phage were screened with pJL8. Three independent phage clones (CHR3, CHR4, and CHR5) were isolated and plaque purified. Unique-sequence subclones pC4B, pC5A, and pC5B were then used to prepare riboprobes for use in screening the jumping library. Three independent jump clones (λH8.1, λH8.2, and λH8.3) were isolated and plaque purified; all three were identified by pC5B. Jump clone inserts were subcloned into pGEM3-blue.

Orientation of the jump clones was determined in two ways. First, the relative orientation of the three jump clones was determined by the presence in all three clones of additional genomic *Eco*RI fragments that are contiguous with pC5B. These extra *Eco*RI fragments presumably result from incomplete *Eco*RI digestion in the construction of the library; because of the presence of these fragments, however, the direction of each jump relative to pC5B could be determined. In addition, the absolute direction of any jump clone can be determined by the crossing of a landmark (e.g., a rare-cutter restriction-enzyme site). Jump clones pCH1, pCH2, and pCH3—representing the jump halves of clones λH8.3, λH8.2, and λH8.1, respectively—were hybridized to CHEF filters that had previously been hybridized with pJL8. One clone, pCH2 (DXS368), was found to cross an *Sfi*I site, as shown in figures 2*A*, 3*B*, and 5. In figure 3*B*, note the different 370-kb *Sfi*I fragment detected by pCH2. This information, combined with the relative-orientation data, allowed the mapping of all three jump clones shown in figure 5. The exact distance of each jump cannot be determined without the cloning of DNA between the jump clones and pJL8.

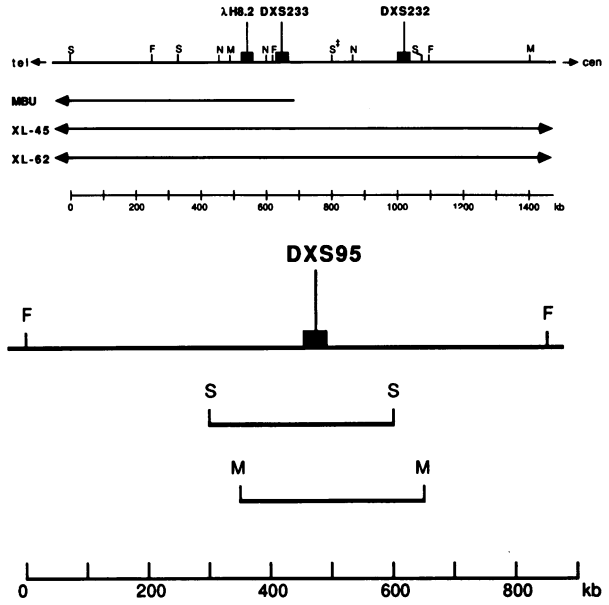


Figure 2 Long-range restriction maps around (*A*) DXS232 and DXS233 and (*B*) DXS95. In *A* the MBU, XL-45, and XL-62 deletions are also illustrated. S = *Sal*I; F = *Sfi*I; M = MluI; N = NarI. The double dagger symbol (‡) denotes the presence of at least one additional *Sal*I site very close to the one indicated. These sites are differentially methylated in normal male and GM1416 cell lines, leading to occasional differences in fragments detected by pJL68 and pJL8.

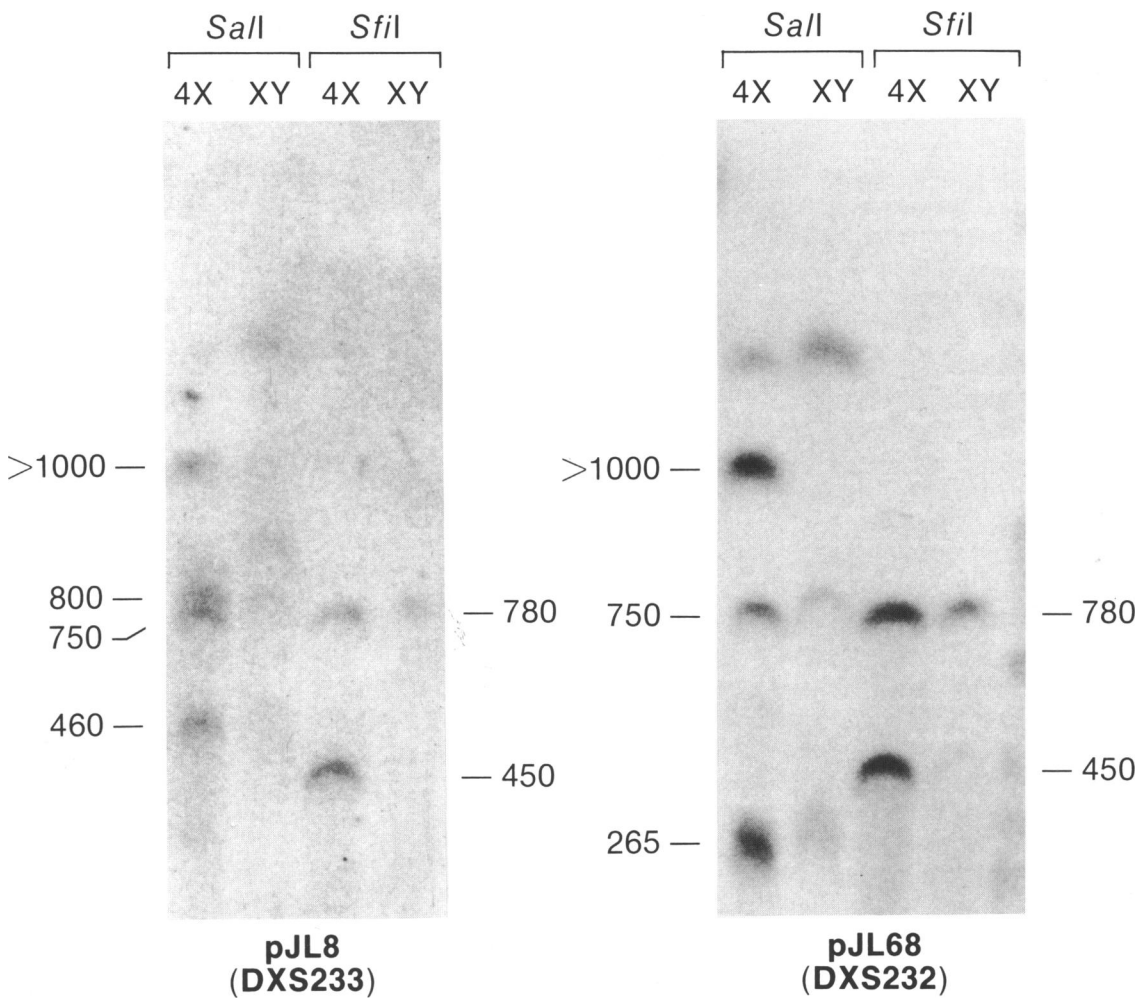
In addition, chromosome walking from phage clones CHR4 and CHR5 has yielded three phage clones (λ2.1, λ16, and λ23.1) that have extended the cloned region around pJL8 to 40–50 kb (fig. 5). All genomic phage clones and all jump clones have been used to screen filters containing DNAs from simple choroideremia probands, in order to identify alterations in these DNAs. In addition, genomic phage clones and jump clones were used to screen "Zoo blots" containing DNAs from a variety of different mammalian species, as the first step in the search for a candidate gene. With none of these clones were any alterations or deletions detected in probands, nor has cross-species hybridization on Zoo blots been identified.

No Proband Alterations Detected on CHEF Southern Blots

Southern blots of CHEF gels containing *Sal*I- and *Sfi*I-digested DNAs from 20 simple choroideremia probands were hybridized with pCH2. This probe detected no alterations in any proband DNAs (data not shown).

Detection of a New Polymorphism with pCH1

All jump clones were used to screen Southern blots of *Eco*RI-digested DNAs from 18 unrelated choroidere-



mia probands. While no alterations are detected by any of these probes, pCH1, a 1.8-kb *Eco*RI/*Ava*I fragment representing the jump half of λH8.3, detects a polymorphism in these *Eco*RI-digested DNAs. This polymorphism was confirmed and the frequency determined by screening Southern filters of *Eco*RI-digested DNAs from 29 unrelated females (representing 58 independent X chromosomes). The 7.8- and 5.6-kb alleles show frequencies of .47 and .53, respectively. These results, in combination with the mapping data shown in table 1, suggest that pCH1 is the closest proximal polymorphic marker to choroideremia.

Discussion

The detailed study of individuals with contiguous gene deletion syndromes with partially overlapping phenotypes due to interstitial deletions of varying extents has been a fruitful approach to mapping and, in some cases, isolating single-gene disease loci. Examples include the Duchenne muscular dystrophy locus (Francke et al. 1985; Ray et al. 1985; Koenig et al. 1987) and the aniridia/Wilms tumor/ambiguous genitalia locus (Davis et al. 1988). Patients with choroideremia and chromosomal abnormalities involving Xq21 are no exception. Patients with large deletions or duplications that include Xq21 have been described with choroideremia and a number of complex phenotypes. For example, patient NP, reported by Tabor et al. (1983) as having choroideremia, psychomotor retardation, and cleft lip and palate, had a large deletion involving most of the Xq21 band that includes DXYS1. X-linked isolated cleft lip and palate has been reported to be linked to DXYS1 in an Icelandic kindred (Moore et al. 1987); this suggests that near the DXYS1 locus there is at least one locus capable of producing this malformation.

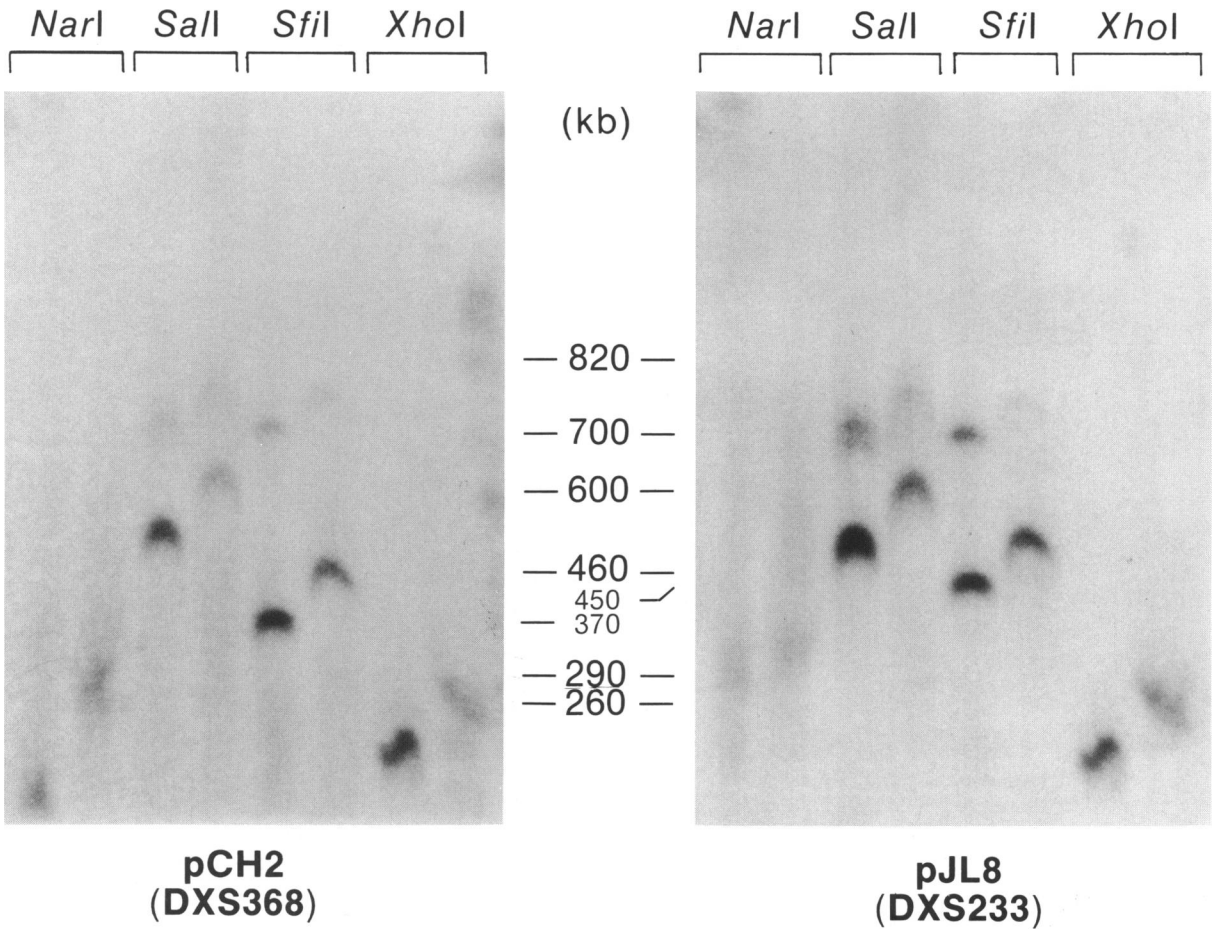


Figure 3 Autoradiographs of CHEF filters sequentially hybridized with (*A*) pJL8 (DXS233) and pJL68 (DXS232) and (*B*) pCH2 (DXS368) and pJL8 (DXS233).

With smaller deletions, a more restricted clinical phenotype is seen. Our results provide information for more precisely defining the localization of the loci for both choroideremia and one form of X-linked mixed deafness.

The choroideremia gene can be placed in the interval defined by the proximal breakpoint in MBU and the distal breakpoint in XL-45, a region that contains DXS233 and DXS95 and that is entirely contained within the deletion in XL-62. We have mapped the MBU proximal breakpoint to a region between DXS232 and DXS233 and to a position distal to the *SalI* site that separates DXS232 and DXS233 (fig. 2*A*). The XL-45 distal breakpoint is not detected by pDP34 (DXYS1), the closest available distal probe. Refinement of this localization comes from our long-range restriction map and from recent data of Cremers et al. (1987), who found that two of eight simple choroideremia patients are deleted for the anonymous DNA sequence DXS165, which is also deleted from XL-45, XL-62, and MBU (Cremers et al. 1989). From this and from their long-range mapping studies, it can be inferred that DXS165 must lie distal to large restriction fragments detected by pJL8 and pCH2. In our studies, probe pCH2 was used to screen for alterations of large restriction fragments in 20 unrelated choroideremia probands. The 700-kb partial *SalI* fragment detected by pCH2 and not by pJL68 (figs. 2, 3) extends in the distal direction, toward the choroideremia gene; no alterations in any subject were seen in this fragment. The findings that no alterations in this fragment are seen in 20 choroideremia probands, while 25% of Cremers et al.'s simple choroideremia patients are deleted for DXS165, suggests that the gene for choroideremia lies distal to the 700-kb *SalI* fragment detected by pJL8 and pCH2.

The deletion of DXS165 in two of eight simple

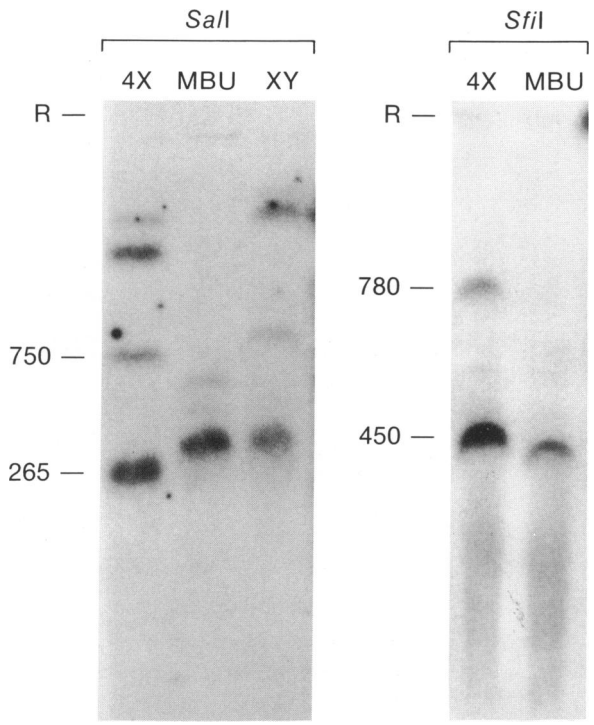


Figure 4 Autoradiographs of two CHEF filters containing *SalI* (*left*) and *SfiI* (*right*) digested DNA from GM1416, MBU, and normal male cell lines, hybridized with pJL68 (DXS232).

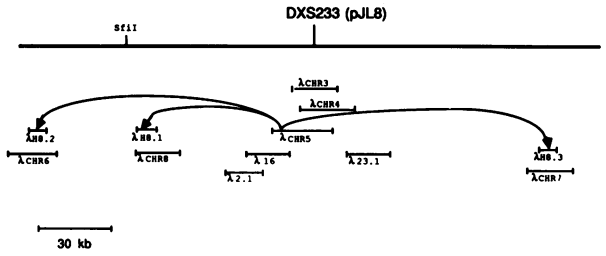


Figure 5 Illustration of chromosome jumping and walking results from DXS233 (pJL8).

choroideremia probands, as well as the absence of any alterations of DXS368 and DXS95, suggests that, of these three markers, the choroideremia gene lies in closest proximity to DXS165. Long-range restriction mapping around DXS165 and DXS95 has not linked these two loci on any common fragment (Cremers et al. 1989). In addition, these two loci are not separated by any deletion or duplication breakpoints, preventing their orientation relative to either the centromere or the choroideremia gene.

Continued chromosome jumping from DXS95 and DXS368 toward the choroideremia gene should provide additional DNA probes, which may be used to refine the long-range restriction map of this region and to allow the orientation of restriction fragments detected by pXG7c (DXS95), pCH2 (DXS368), and p1bD5 (DXS165). These additional probes can also be used to detect alterations of large restriction fragments in simple choroideremia probands and may facilitate the cloning of the choroideremia gene from preparative pulsed-field gels.

X-linked mixed deafness with stapes fixation and perilymphatic gusher (DFN3; MIM 30440) is a rare form of deafness characterized by congenital hearing loss with both sensorineural and conductive components (Nance et al. 1971; Thorpe et al. 1974; Cremers et al. 1985). At tympanotomy and stapedectomy, marked leakage of perilymph occurs from the inner ear. Recent reports have shown linkage of this form of congenital deafness to DXYS1 (Wallis et al. 1988) and to PGK (Brunner et al. 1988). Thus, in kindred XL-62 reported here, the congenital mixed (sensorineural and conductive) deafness with stapes fixation and perilymphatic gusher that was documented in association with choroideremia, mental retardation, and an interstitial deletion of Xq21 is very likely to be DFN3, on both phenotypic and genetic grounds. Probands in XL-45 also show congenital mixed deafness with both sensorineural and conductive deficits in the absence of middle-ear effusion or other causes of conductive hearing loss. Even though no operative procedures were performed on these probands, the similarity of this phenotype to that seen in XL-62 probands suggests a diagnosis of DFN3 in the XL-45 kindred as well. In contrast, the MBU proband has normal hearing. The locus for DFN3 can therefore be placed more accurately within the XL-45 deletion region that is proximal to and excluded from the deletion in MBU, a region that is flanked by markers DXS233 and by the more distal of DXS276 and DXS72 (table 1). Flow-karyotype analysis places an upper limit on the size of this region at 5,300 kb, the entire XL-45 deletion. However, since the deafness locus is excluded from the region of overlap between XL-45 and MBU—a region that contains DXS165 and DXS95 (Cremers et al. 1989), both of which are located on large nonoverlapping restriction fragments—the area in which to search for the stapes fixation deafness gene should be considerably smaller than this upper estimate. Continued chromosome jumping in the region flanked by the MBU and XL-45 proximal breakpoints may provide sequences involved in this form of congenital deafness.

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